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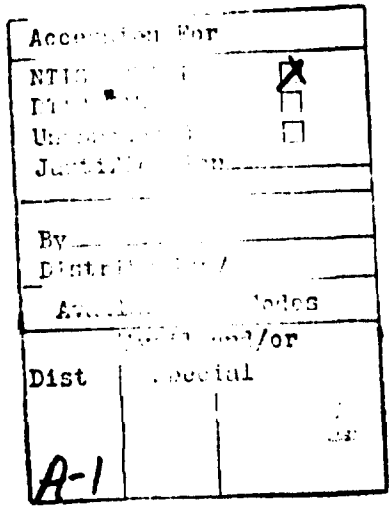

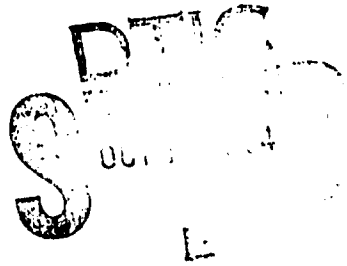

PAPER NO. 20

TOXICOLOGY OF NATURAL AND MAN-MADE TOXICANTS IN DRINKING WATER

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INTRODUCTION

A chemical is thought to modify the toxic effects of a second chemical by one of four general mechanisms. Most often considered are pharmacologic interactions, or those situations in which there is a commonality in the receptor through which the toxic chemicals exert their effects. The net result of the interaction depends upon the relative affinity of the chemicals (or their active products) and whether the chemicals act as agonists or antagonists. The second type of interaction is that in which a chemical modifies the uptake, distribution, metabolism, or excretion of a toxic chemical. Third are interactions that depend upon the activation or inhibition of physiologic systems that compensate for or amplify the effects of a second chemical. Finally, there are the cases where one chemical actually reacts with a second chemical within the body to produce a more or less toxic group of chemicals.

The present paper deals with an example of the last type of interaction, that of chemical reaction. Disinfection of drinking water utilizes reactive chemicals, usually oxidizing agents, that react with biochemical components of bacteria, viruses, and parasites to kill these infectious agents. The effectiveness of this approach has been demonstrated by the marked decrease in waterborne infectious disease in this country since the early 20th century. However, it is becoming increasingly apparent that disinfectants also react with organic materials present in water or in the gastrointestinal tract to produce potentially toxic byproducts. This was first realized when it was found that chlorination of drinking water gave rise to trihalomethanes (Bellar and Lichtenberg, 1974; Rook, 1974). This area has become increasingly complex in the last 2-3 years as diversity of substrates that are available for reaction has become recognized. As might be suspected, the diversity of toxicologic properties associated with these reactions is also growing. For the purposes of this paper, however, only reactions of chlorine to produce chemicals that possess carcinogenic and mutagenic properties will be considered.

REACTION OF CHLORINE WITH ORGANIC CHEMICALS IN DRINKING WATER

Meier et al. (1983) demonstrated that treatment of humic acid with chlorine produced direct acting mutagens in the standard plate assay of Ames (1975) utilizing Salmonella tester strains. In Table 1 the effect of varying pH on mutagen formation is compared. Mutagen formation and chlorine substitution, indicated by formation of total organic halogen (TOX), is very much favored when the pH is allowed to drift to acid pH following addition of chlorine. Although there is still considerable TOX formed at alkaline pH, the mutagenic activity is sharply reduced. This circumstance is at least partially attributable to the fact that mutagens formed at acid pH are very alkaline labile (Meier et al., 1983). It is notable that trihalomethane formation is favored at alkaline pH and they would not be detected under the test conditions used because of their volatility.

TABLE 1. COMPARISON OF THE pH DEPENDENCE OF MUTAGEN AND CLASTOGEN FORMATION UPON TREATMENT OF HUMIC ACID WITH CHLORINE (HOCl/OCl)

No.	Sample	Tox	Mutagen Activity[a] TA98	TA100
A	Chlorinated Humic (pH: 7.0 -2.8)	414	339 ± 29 (100)	1696 ± 148 (100)
B	Chlorinated Humic (pH: 7.0 - 6.5)	263	62 ± 10 (18)	367 ± 34 (22)
C	Chlorinated Humic (pH: 11.5 - 6.5)	280	N.S.[b] (15)	490 ± 33 (29)
D	Non-chlorinated	0.3	N.S.[b] (15)	N.S.[b] (15)

[a] Net revertants/ml of sample, calculated from the linear portion of dose response curve. Numbers in parentheses indicate the percent of activity in sample A.

[b] N.S. = Not significant (i.e., less than 2-fold above background) for this experiment. Negative control values were 21 revertants/plate for TA98 and 98 revertants/plate for TA100; the highest dose level was 0.4 ml. Therefore, a N.S. response would be less than 52 revertants/ml for TA98 and less than 245 for TA100.

Mutagenic activity formed is proportional to the degree of chlorine substitution as determined with total organic halogen analyses at chlorine to carbon molar ratios between 0 to 1.0. It is difficult to determine the extent to which this is only a fortuitous relationship since the relative importance of oxidation reactions versus chlorine substitution reactions of

$HOCl/OCl^-$ in the production of mutagenic activity has not been established. Interpretation is very clouded by the fact that mutagens formed are very alkaline labile.

More than 40 compounds have been tentatively identified in these reaction mixtures (Coleman et al., 1984). They fall into several classes, the trihalomethanes, chlorinated alkanes, alkenes, aldehydes, ketones, acids, nitriles, and aromatics (Table 2). Among these chemicals are a number of established carcinogens and mutagens and others which have been demonstrated to possess such properties in our own laboratory.

TABLE 2. TENTATIVE IDENTIFICATION OF MAJOR CONSTITUENTS OF CHLORINATED HUMIC ACID[a]

TRIHALOMETHANES

Chloroform[c]

ACIDS

Dichloroacetic Acid
Trichloroacetic Acid

ALDEHYDES

Dichloroacetaldehyde
Trichloroacetaldehyde
Dichloropropanal
Trichloropropanal
2-Chloropropenal
2,3-Dichloropropenal
3,3-Dichloropropenal[b]
2,3,3-Trichloropropenal
Trichlorobutanal
Dichlorobutenal

KETONES

1-Chloro-2-Propanone
1,1-Dichloro-2-Propanone[b]
1,3-Dichloro-2-Propanone[b]
1,1,1-Trichloro-2-Propanone[b]
1,1,3-Trichloro-2-Propanone
1,1,1,3-Tetrachloro-2-Propanone
1,1,3,3-Tetrachloro-2-Propanone
Pentachloropropanone[b]
3-Chloro-2-Butanone
1,1,1-Trichloro-2-Butanone
1,1,3-Trichloro-2-Butanone

KETONES (Cont.)

1,3-Dichloro-2-Butanone
1,1-Dichloro-2-Butanone
3,3-Dichloro-2-Butanone
1-Chloro-3-Buten-2-One
3-Chloro-3-Buten-2-One
Dichloro-3-Buten-2-One
Trichloro-3-Buten-2-One
Tetrachloro-3-Buten-2-One
Pentachloro-3-Buten-2-One
Trichlorocyclopentenedione

NITRILES

Chloroacetonitrile[c]
Dichloroacetonitrile[b]
Trichloroacentonitrile[c]
Dichloropropenenitrile
Dichloropropenentrile
Trichloropropenenitrile

AROMATICS

2,4,6-Trichlorophenol
Trichlorodihydroxy Benzene
Tetrachlorothiophene

ALKANES AND ALKENES

Hexachloroethane[c]
Pentachloropropene[b]
Tetrachlorocyclopropene
Hexachlorocyclopentadiene

[a] Data obtained from Coleman et al. (1984).
[b] Chemicals identified as mutagens
[c] Chemicals identified as carcinogens

Despite the fact that a number of mutagenic chemicals have been identified, to this point only about 1.5% of the total mutagenic activity can be accounted for by chemicals for which both quantitative analyses and mutagenic characterization have been possible. Admittedly, this is somewhat of an artifact due to the lack of appropriate standards and the time lag associated with getting these chemicals synthesized and tested. Nevertheless, this figure does provide some estimation of the complexity of the overall problem. Adding to this complexity is that the formation of mutagenic activity and the identity of products can be markedly changed by incorporating trace quantities of Br^- into the reaction mixture. As Br^- is increased to a molar ratio of 0.1 to the chlorine added, there is an approximate doubling of the mutagenic activity (Meier, unpublished observations). As would be predicted from the well-known ability of HOCl to activate Br^- to HOBr, bromination reactions begin to predominate. As can be seen in Table 3, brominated compounds become very evident at ratios of Br^- to HOCl of 0.01 and progressively increase until completely brominated compounds predominate at ratios above 0.1.

TABLE 3. COMPOUNDS IDENTIFIED[a] IN METHYLENE CHLORIDE EXTRACTS OF HUMIC ACIDS TREATED WITH CHLORINE[b] WITH VARYING CONCENTRATIONS OF BROMIDE (BR^-)[c]

Compounds	Molar Ratio of Br^-/C Where Compound Concentration Is Highest[d]
1,1,1-Trichloro-2-Propanone[e]	0
Pentachloro-2-Propanone[e]	0
Chloroform	0.01
Trichloroacetonitrile	0.01
Bromodichloromethane	0.01
Dichloroacetonitrile	0.01
1,1-Dichloro-2-Propanone	0.01
Bromochloromethane	0.05
Bromochloroacetonitrile	0.1
Dibromochloromethane	0.1
Bromochloroacetonitrile	0.1
1,1,1-Bromodichloro-2-Propanone[e]	0.1
1,1,1-Dibromochloro-2-Propanone[e]	0.1
Bromoform	0.5,1
Tetrabromomethane	1
1,1,1-Tribromo-2-Propanone[e]	1

[a] 60M x 0.25 MM I.D. DB1 fused Silica column
[b] Cl:C Molar ratio 1:1
[c] Data obtained from Coleman et al. (1984)
[d] Applicable only to ratios tested, concentration is RIC area
[e] Standard not available, identified by visual interpretation of mass spectrum

BY-PRODUCTS OF CHLORINE PRODUCED IN VIVO

Drinking water in the U.S. is treated to a residual. This means that water consumed by the public would usually contain between 0.5 and 2 mg/L of disinfectant. It is obvious that the variety of potential substrates for chlorine available within the gastrointestinal tract adds another dimension to this problem. The question naturally arises "can interactions of chlorine reactions with substrates present .. the GI tract result in products with carcinogenic and mutagenic properties?"

Experiments sponsored by our laboratory show that OCl^- and $ClNH_2$ administered orally increase spermhead abnormalities in B6C3/F_1 mice (Meier et al., 1983). An increased percent of abnormal sperm was seen at 3 weeks following dosing, but not at 1 and 5 weeks, consistent with an effect at the spermatocyte stage of spermatogenesis. HOCl was without effect in this system. It is unlikely that OCl^- is capable of reaching the testis because of its reactivity. Therefore, it is felt that these data are evidence for the formation of highly mutagenic chemical(s) in the gastrointestinal tract. It should be noted that conditions of pH responsible for the formation of mutagens in vivo are the opposite of those observed in reactions with humic acid. The fact that the same result was observed in two experiments, with OCl^- (pH 8.5) and with $ClNH_2$ (pH 9), indicates a degree of consistency in the results.

To document the extent to which chlorination by-products can be formed in the gastrointestinal tract, Mink et al. (1983) intubated rats with relatively high doses of OCl^- (ca. 150 mg/g) and observed the appearance of by-products in the stomach contents and plasma. Table 4 lists the products identified by GC/MS when animals were treated with a chlorine solution containing Br^-. Within the stomach contents, many of the same products seen in the reactions with humic acids were identified but could not be meaningfully quantitated. Primarily, we saw halomethanes, haloacetonitriles, and halogenated acetic acids.

To what extent can the spermhead abnormalities be accounted for by these products? Not at all, it would appear. Topham (1980) demonstrated that chloroform is negative in this bioassay. Our laboratory has tested the haloacetonitriles identified in these experiments in the mouse spermhead assay at much higher doses than would be expected to be formed in the G-I tract and found that they were negative as well (Bull et al., unpublished results). Although we have not yet tested this possibility, it seems highly unlikely that these effects could be attributed to the highly polar haloacetic acid products formed both because of a lack of intrinsic activities in other mutagenesis test systems and on pharmacokinetic grounds.

TABLE 4. HALOGENATED PRODUCTS OBSERVED IN THE RAT STOMACH AND BLOOD PLASMA FOLLOWING ACUTE TREATMENT WITH CHLORINE AND KBr[a]

Compound	Stomach Content	Plasma
Chloroform	+[b]	-
Bromoform	+	+
Bromodichloromethane	+	-
Dibromochloromethane	+	+
Dichloroacetonitrile	+	-
Trichloroacetonitrile	-	-
Dibromoacetonitrile	+	-
Dichloroacetic Acid	+	-
Dichloroacetic Acid	+	-
Trichloroacetic Acid	+	-
Dibromoacetic Acid	+	-
Dibromomethane	+	-

[a] Treatment involved gavage with total dose of 48 mg Cl (as NaOCl) and 32 mg Br^- (as KBr). Analyses conducted 1 h after dosing.

[b] Positive sign (+) indicates identification of the indicated compound by GC/MS. Negative sign (-) indicates compounds were below detection limit.

However, it is clear that the haloacetonitriles do possess significant biological properties (Table 5). Two haloacetonitriles, dichloroacetonitrile and bromo-chloroacetonitrile, are mutagens in Salmonella. In addition, the dibromo-, the bromochloro-, the trichloro-, and the monochloro- acetonitriles act as tumor initiators in the mouse skin (Bull et al., unpubl. results). The ability of these same four compounds to produce lung adenomas has also been demonstrated in Strain A mice with oral administration of 10 mg/kg 3 times weekly for 8 weeks.

CONCLUSIONS

The major conclusion of this work is that toxic properties associated with reactive chemicals such as the drinking water disinfectants must be viewed as being potentially quite diverse. It is clear that a diversity of mutagenic and carcinogenic chemicals in addition to the trihalomethanes are formed both in drinking water and in vivo through reactions of chlorine with endogenous substrates. Evaluation of the overall toxic effects of chlorine must deal with the diversity of products that can be formed and the fact that these products have their own intrinsic toxicologic properties. To complete the situation, it is also quite clear that the nature of the products formed will vary with the substrates available in the diet. Amino acids have been shown as substrates that can give rise to haloacetonitriles

TABLE 5. ACTIVITY OF HALOACETONITRILES IN MUTAGENESIS AND CARCINOGENESIS SCREENING PROCEDURES

	Salmonella[a]	SCE Induction[a]	Tumor Initiation Mouse Skin[b]	Mouse Lung Adenomas[c]
Chloroacetonitrile	-[d]	+	+	++
Dichloroacetonitrile	++	±	±	±
Trichloroacetonitrile	--	++	+	+
Dibromoacetonitrile	--	+++	+++	+
Bromochloroacetonitrile	+++	+++	++	+++

[a] Meier et al., Personal Communication
[b] Bull et al. (1982[b]) Toxicologist 2:326
[c] Bull et al. (1983) Unreported Data
[d] Indication of negative (-) or relative degree of positive response (+)

(Biebar and Trehly, 1982). They may also give rise to a group of organic chloramines (Scully et al., 1983), an example of which is N-chloropiperidine, extremely potent in producing spermhead abnormalities in mice.

In summary, reaction of chlorine in biological systems will necessarily give rise to a very complex mixture of organic chemicals. Whether the toxic chemicals that arise from reactions of chlorine are produced in sufficient quantities to present a meaningful hazard to human health remains to be determined. However, by-products of chlorination are the most common and plentiful organic chemicals identified in drinking water. Their levels outstrip the levels of industrial chemicals by 1-2 orders of magnitude in finished waters taken from surface sources. Finally, we must recognize that other potential disinfectants are also reactive chemicals (chloramine, chlorine dioxide, and ozone). Therefore, analogous problems will also arise from their use. The only resolution of this problem is a systematic study of the reactions of these chemicals, identification of the major by-products, and characterization of their toxicologic properties. Once these are established, the toxicity of these extremely complex mixtures can be meaningfully and usefully studied.

REFERENCES

Ames, B. N., J. McCann, and E. Yamasaki (1975), Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test, Mutat. Res., 31:347-363.

Bellar, T. A., J. J. Lichtenberg, and R. C. Kroner (1974), The occurrence of organohalides in chlorinated drinking waters, J. Am. Water Works Assoc., 66:703-706.

Biebar, T. I. and M. L. Trehy (1983), Dihaloacetonitriles in chlorinated natural waters, In: (Jolley et al., eds.) Water Chlorination: Environmental Impact and Health Effects, pp. 85-96.

Bull, R. J., M. Robinson, R. D. Laurie, and H. P. Ringhand (1982), Tumor initiating activity of haloacetonitriles, by-products of chlorination of drinking water, Pharmacologist, 24:326.

Coleman, W. E., J. W. Munch, W. H. Kaylor, R. P. Streicher, H. P. Ringhand, and J. R. Meier, GC/MS analysis of mutagenic extracts of aqueous chlorinated humic acids - A comparison of the by-products to drinking water contaminants, Environmental Science and Technology, In press.

Meier, J. R., R. J. Bull, and M. C. Cimino (1983), Activity of drinking water disinfectants in in vivo tests of mutagenic potential, Pharmacologist, 25:468.

Meier, J. R., R. D. Lingg, and R. J. Bull (1983), Formation of mutagens following chlorination of humic acid: A model for mutagen formation during drinking water treatment, Mutation Research, 118:25-41.

Mink, F. L., W. E. Coleman, J. W. Munch, W. H. Kaylor, and H. P. Ringhand (1983), In vivo formation of halogenated reaction products following peroral sodium hypochlorite, Bull. Environ. Contamin. Toxicol., 30:394-399.

Rook, J. J. (1974), Formation of haloforms during chlorination of natural waters, Water Treat. Examin., 23:234-243.

Scully, F. E., Jr. and M. A. Bempong (1982), Organic N-chloramines: Chemistry and Toxicology, Environmental Health Perspectives, 46:111-116.

Topham, J. C. (1980), Do induced spermhead abnormalities in mice specifically identify mammalian mutagen: rather than carcinogens? Mutat. Research, 74:379-387.